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IAM Chromatography: An *in Vitro* Screen for Predicting Drug Membrane Permeability

Charles Pidgeon,*,† Shaowei Ong,† Hanlan Liu,† Xiaoxing Qiu,† Mary Pidgeon, Anne H. Dantzig,‡ John Munroe,‡ William J. Hornback,‡ Jeffery S. Kasher,‡ Louis Glunz,§ and Ted Szczerba§

Department of Medicinal Chemistry, School of Pharmacy, Purdue University, West Lafayette, Indiana 47907, Lilly Research Labs, Lilly Corporate Center, Eli Lilly and Company, Indianapolis, Indiana 46285, and Regis Chemical Company, 8210 Austin Avenue, Morton Grove, Illinois 60053

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Fluid cell membranes are the main barrier to drug absorption when diffusion limits uptake. Immobilized artificial membranes (IAMs) are solid phase models of fluid membranes that predicted oral drug absorption in mice for a homologous set of cephalosporins. IAMs also predicted drug permeability through Caco-2 cells. Since drug permeability in Caco-2 cells is known to correlate with the oral absorption of drugs in humans, IAMs may also model drug absorption in humans. IAM analysis is experimentally simple, and large-volume screening of experimental compounds for drug absorption is possible.

With the current emphasis on home managed care, noninvasive methods of drug delivery are a highly desired property of new drug candidates. Oral drug delivery is the preferred route of drug administration, and screening experimental compounds for oral drug absorption in animals requires extensive experimental effort. Both the experimental difficulty and costs associated with animal studies prevent the evaluation of many compounds during the drug discovery process. To circumvent the problems associated with animal models, a number of *in vitro* models for predicting oral drug absorption have been developed.[1-5] The drug discovery process usually requires evaluating many compounds for their oral absorption, and *in vitro* models facilitate the screening of compounds for their potential absorption when given orally.

It is well-known that the major absorption barrier to drugs given orally is the gastrointestinal cell membranes and that most drugs given orally are absorbed across the intestinal mucosa by a passive diffusion mechanism.[4] Thus the cell membrane (Figure 1A) comprises the main "biological barrier" for drug diffusion that results in the absorption of most commercially available drugs (Figure 1B). Equilibrium drug partitioning into fluid membranes, K_m, is frequently a rate-limiting step in drug absorption, and liposomes have been used as a model to measure K_m. Immobilized artificial membranes (IAMs) shown in Figure 1C are a model of liposome membranes, and drug partitioning into liposome membranes was accurately predicted using IAMs.[6] Since IAMs predicted drug partitioning into fluid liposome membranes, we tested the ability of IAMs to predict drug transport through membranes.

Permeation, P_m, of a drug through membranes is directly proportional to K_m[7]

$$P_m = \frac{D_m K_m}{L} \quad (1)$$

where D_m is the membrane diffusion coefficient of the solute and L is the membrane thickness (~30 Å for the hydrocarbon domain of the bilayers). Although K_m is the major source of variation to drug permeability, passive drug diffusion through cell membranes also depends on D_m. Furthermore, D_m significantly depends on the molecular size or molecular weight of the drug. As molecular weight increases, D_m dramatically decreases such that very few if any commercially available drugs have high molecular weights.[7,8] In fact, most drugs given orally are smaller than the size of membrane lipids that create the barrier to drug absorption (Figure 1B), and the majority of drugs exhibit sufficient lipophilicity to allow membrane permeability. Figure 1B reflects an enormous amount of current research dollars, and ignoring the molecular weight dependence of drug absorption has contributed in part to the number of false starts in the drug discovery process. False starts in drug discovery are frequently the result of side effects or toxicity, but ignoring the molecular weight dependence of drug absorption may also lead to false starts.

Chromatographic models to predict drug absorption are experimentally much easier than cell culture models and animal models. IAMs are a chromatographic model of the membrane lipid environment of cells.[9-11] As shown in Figure 1C, the head group of the membrane lipids are depicted as balloons tethered to a "hydrocarbon string". Octadecyl (ODS) reversed phase chromatography columns have been used as models, but ODS surfaces contain only hydrocarbon chains; consequently, ODS surfaces can only model octanol/water partitioning. Because IAMs contain the polar head group of lipids as the first contact site between the drug and the surface, the IAM surface is obviously a better chromatographic model of the membrane lipid barrier than the ODS surface. We note that mobile phase optimization is routine for ODS chromatography to obtain the best correlations; however, optimization is not performed

* Address correspondence to Dr. C. Pidgeon: phone, 317-494-6251; fax, 317-494-6790; email, pidgeon@mace.cc.purdue.edu.
† Purdue University.
‡ Lilly Research Labs.
§ Regis Chemical Company.
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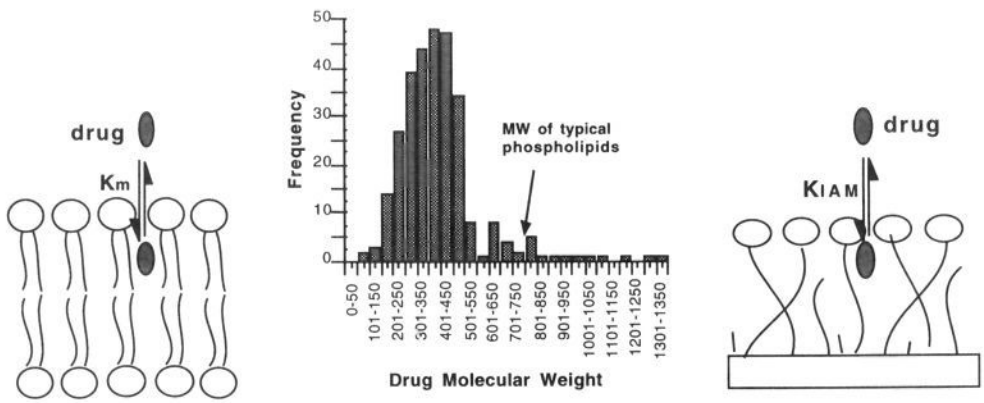
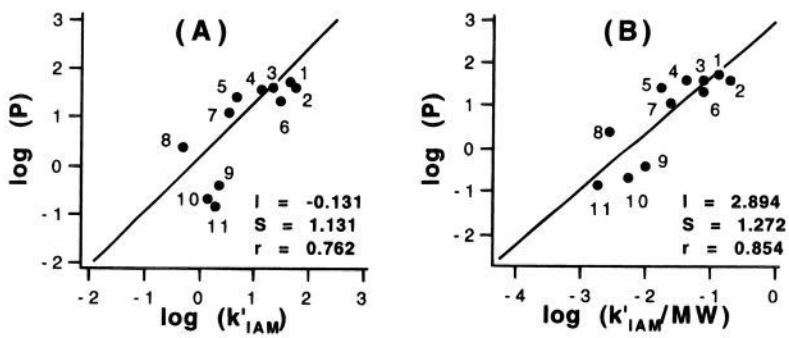


Figure 1. (A) The phospholipid bilayer is the main barrier for the diffusion of drugs across absorption barriers found in the gastrointestinal tract. (B) A histogram showing the molecular weight distribution of ~400 commercially available oral drugs. When the size of a compound is larger than phospholipid molecules, the compound is not absorbed to any significant extent unless active transport processes exist for the compound. Drugs that have molecular weights above ~750 Da are high molecular salts. (C) IAMs are intended to emulate cell membranes. Immobilized phospholipids are depicted as balloons tethered to a string. The strings without balloons near the silica floor are C10 and C3 alkyl groups.



1. corticosterone (364)
2. propanolol (259)
3. alprenolol (249)
4. warfarin (308)
5. metoprolol (267)
6. hydrocortisone (362)
7. salicylic acid (138)
8. acetylsalicylic acid (180)
9. terbutaline (225)
10. atenolol (266)
11. arginine-vasopressin (1071)

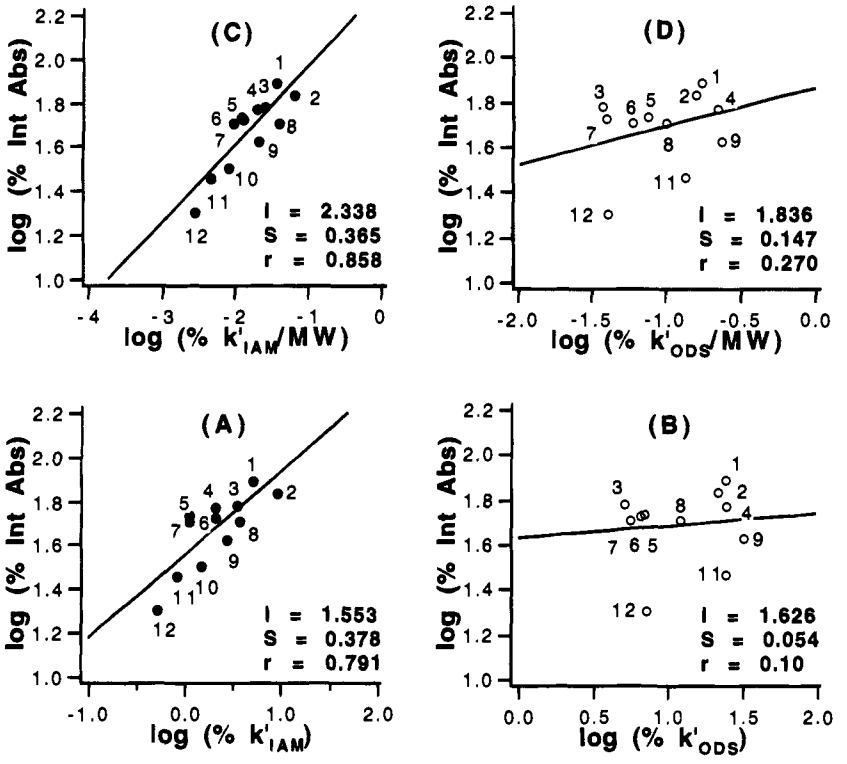
Figure 2. Correlation of drug intestinal permeability (P_m) through Caco-2 cells with drug partitioning to ^{ether}IAM.PC$^{C10/C3}$ columns (log k'_{IAM}). Figure 2A shows that log P_m correlates with log k'_{IAM} with $r = 0.740$. Figure 2B shows that replotting the data after correcting for the dependence of D_m on molecular weight improves the correlation to $r = 0.854$. MW are given in parentheses after the chemicals. P_m was measured by Artursson et al.[4] The retention times (t_R, in minutes) on IAM were measured on a 15 cm × 0.46 cm ^{ether}IAM.PC$^{C10/C3}$ column using a mobile phase of 0.01 M PBS buffered at pH 7.4. k'_{IAM} was calculated from $k'_{IAM} = (t_R - t_o)/t_o$, where t_o is the retention time of an unretained compound, i.e., citric acid. Complete details of IAM chromatography are available.[6]

with the IAM analysis because 100% aqueous mobile phase is used to elute the compounds. For compounds with long retention times, an extrapolation method is used as described below.

Regarding nonchromatographic models, the human intestinal Caco-2 cell line has provided an *in vitro* cellular epithelium model to predict the intestinal permeability of drugs.[2-5] Artursson et al.[4] have shown that drug permeability in the Caco-2 cell model can be used to predict drug absorption in humans, and thus the Caco-2 cell model can be used to screen for drug absorption prior to clinical trials. Figure 2A shows the correlation of the logarithm of the capacity factors (k'_{IAM}) of 11 drugs measured on an ^{ether}IAM.PC$^{C10/C3}$ column with the logarithm of the intestinal permeability coefficients (P_m) of the 11 drugs through Caco-2 cells as measured by Artursson et al.[4,29] For this group of 11 structurally diverse drugs, log k'_{IAM} correlates with log P_m with a linear correlation coefficient $r = 0.762$. Although the capacity factor k'_{IAM} is directly proportional to K_m,[6] k'_{IAM} does not model drug diffusion D_m in the membrane. D_m depends on molecular weight (MW), and D_m is often corrected for the effect of molecular size on diffusion according to[16]

$$D_m \propto 1/V^n \qquad (2)$$

where V is the molar volume of the drug molecule and n is a constant. Assuming the MW is proportional to V and n is set to 1, then to a first approximation D_m is inversely proportional to 1/MW. Converting k'_{IAM} to k'_{IAM}/MW and replotting the data in Figure 2A significantly improves the correlation $r = 0.854$ (Figure 2B). On the basis of Figure 2, drug partitioning to IAMs, as



1. m-nitroaniline (138.1)
2. p-nitroaniline (138.1)
3. salicylic acid (138.1)
4. p-toluidine (107.2)
5. aniline (93)
6. m-nitrobenzoic acid (167.1)
7. benzoic acid (94.0)
8. phenol (122.0)
9. acetanilide (135.2)
10. antipyrine (188.2)
11. theophylline (180.2)
12. acetylsalicylic acid (180.2)

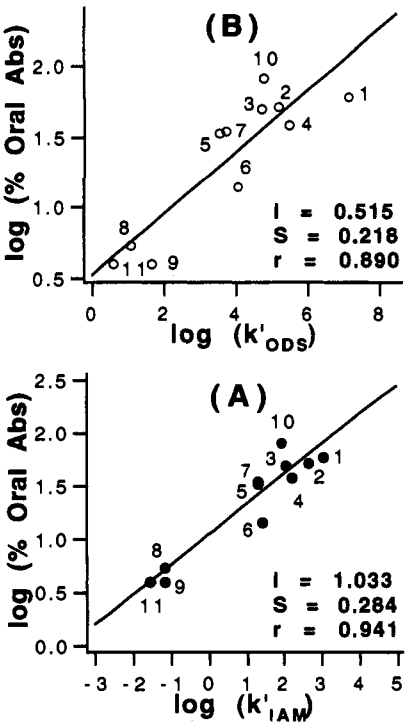
Figure 3. Correlation of rat intestinal drug absorption with drug partitioning to an ^{ether}IAM.PC$^{C10/C3}$ column (log k'_{IAM}) or ODS column (log k'_{ODS}). log (% int abs) correlates with log k'_{IAM} with $r = 0.791$ (A), but does not correlate with log k'_{ODS}, $r = 0.1$ (B). Parts A and B of Figure 3 show the data replotted after correcting for the effect of molecular weight on D_m. MW is given in parentheses after the chemicals. The intestinal absorption of the drugs from rat small intestine were measured by Schanker et al.[1] and the effective pH at the surface of the intestinal epithelial is about 5.4.[1,26] IAM capacity factors, k'_{IAM}, were measured on a 15 cm × 0.46 cm ^{ether}IAM.PC$^{C10/C3}$ column using a mobile phase of 0.01 M PBS buffered at pH 5.4. The ODS capacity factors, k'_{ODS}, were measured using the same aqueous mobile phase; however, a 3 cm × 0.46 cm column was used because the retention times were very long and a shorter ODS column (relative to the IAM column) was needed to elute the solutes in a reasonable amount of time.

measured chromatographically, correlates very well with the intestinal permeability of drugs measured in the Caco-2 cell model, particularly if corrections for the size of the drug molecules are made.

To further evaluate the usefulness of IAM chromatography, a comparison was made of 12 drugs evaluated by Schanker et al. using a perfused rat small intestinal model[1] which measures the percent absorption (% int abs) at the actual tissue site. For this group of structurally diverse molecules, the correlation of log (% int abs) vs log k'_{IAM} was $r = 0.791$ (Figure 3). For comparison, the accepted ODS chromatographic method for measuring lipophilicity was also used and a correlation of $r = 0.10$ was obtained using the same aqueous mobile phase (Figure 3B). Correcting for the dependence of D_m on molecular weight, both the IAM column (Figure 3C) and ODS column (Figure 3D) have improved correlations, but the ODS model still gives such a poor correlation that it is not useful for predicting drug absorption.

Using 11 structurally related cephalosporin analogs, IAM chromatography also predicted oral absorption in mice. Figure 4A shows that log k'_{IAM} correlates well with log (% oral abs) with $r = 0.941$, whereas an ODS column gave a correlation of $r = 0.890$ (Figure 4B). Although IAM chromatography is slightly better than ODS chromatography, the key finding is that the evaluation of drugs by IAM chromatography is much easier than ODS chromatography. This is because the capacity factor data in Figure 4 are for mobile phase conditions that are completely aqueous. Some compounds require acetonitrile for elution, and the capacity factors from several isocratic elutions at different acetonitrile concentrations were necessary. Linear extrapolation of plots of log (capacity factor) vs log (% acetonitrile) gives the capacity factor at 0% acetonitrile.[17] This extrapolation method was needed for 9 of the 11 drugs on the ODS column and only 2 of the 11 drugs on the IAM column. Thus the IAM column not only gives a better correlation than ODS columns (Figure 4), but IAM chromatography usually does not require the tedious data collection needed for extrapolation to 0% acetonitrile.

An important finding from this study and our earlier work[6] is that IAM chromatography always gives better correlations than ODS chromatography or octanol/water partitioning systems regarding (i) the prediction of solute transport through any biological barrier, and (ii) modeling the partitioning of drugs to fluid membranes. In support of this, the intestinal transport of 11 struc-



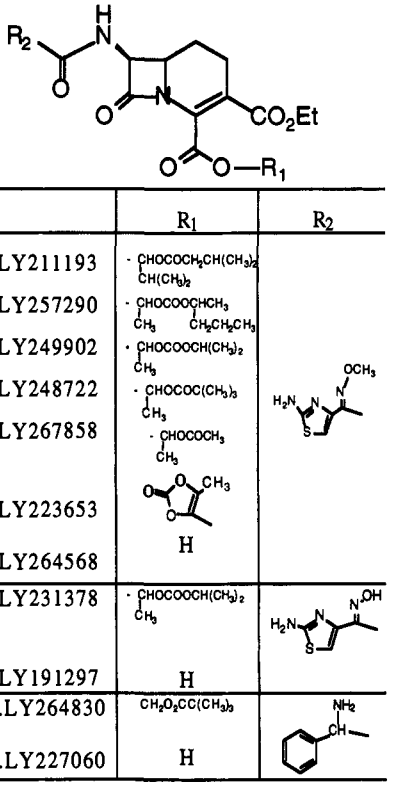


Figure 4. Correlation of oral drug absorption in mice with drug partitioning to an ^{ether}IAM.PC$^{C10/C3}$ column (log k'_{IAM}) or ODS column (log k'_{ODS}). k'_{IAM} and k'_{ODS} were measured on a 3 cm × 0.46 cm ^{ether}IAM.PC$^{C10/C3}$ column and a 3 × 0.46 cm ODS column, respectively, using a mobile phase of 0.01 M PBS buffered at pH 7.4 except for compounds **1** and **2** for ^{ether}IAM.PC$^{C10/C3}$ column and compounds **1**–**8** and **10** for ODS column, which did not elute with an aqueous phase. For the compounds not eluting with an aqueous mobile phase, four concentrations of acetonitrile were used as isocratic mobile phases and linear plots of log k'_x vs x were extrapolated to the x coordinate to obtain k'_{IAM} or k'_{ODS} that theoretically corresponds to 0% acetonitrile. All data given represent k' values corresponding to 100% aqueous mobile phases. Oral absorption of these drugs was measured as described.[27,28]

turally unrelated drugs (Figure 3) was predicted by IAM chromatography but not ODS chromatography. The oral absorption of 11 structurally similar drugs (Figure 4B) gave acceptable correlations when modeled on either ODS or IAM columns, but IAM chromatography gave a better correlation, and in addition, the experimental data was much easier to obtain. Our previous studies demonstrated that (i) for structurally similar hydrophobic drugs, IAM and ODS chromatography as well as octanol/water partition models correlate well with the membrane partition coefficient K_m, but (ii) for structurally nonrelated compounds, K_{oct} does not correlate with K_m and/or k'_{IAM}.[6] A review of chromatographic methods that are used to model biological partitioning has been recently published.[18]

On the basis of our previous studies that IAM surfaces correlates with other *in vitro* methods such as liposome partitioning,[6] the present study demonstrates that IAM surfaces correlate with biological methods. For example, drug partitioning into IAM surface was predictive of oral drug absorption in mice for a series of cephalosporin analogs. Furthermore, partitioning into IAM surfaces correlated well with the permeability of Caco-2 monolayers for a group of structurally diverse clinically used drugs. Moreover, the permeability of Caco-2 cells for these compounds had been previously shown to be predictive of oral absorption in humans.[4] Thus, IAMs may be useful as an early predictor of drug absorption in humans. In addition to bioavailability predictions, drug pharmacokinetic parameters correlate very well drug log k' values obtained on IAM columns.[19]

Limitations of IAM Chromatography in Predicting Bioavailability

Drug absorption is a complex phenomena. Numerous factors contribute to the fraction of the administered dose that reaches the blood. Factors that decrease the absorption of drugs include poor dissolution of the compound, drug precipitation at the absorption site, chemical and bacterial degradation at the absorption site, and the first pass metabolism in the intestinal cells and the liver. Incorporating all of these factors into a single model for predicting drug absorption is virtually impossible. However, the ideal starting point for drug formulation should be to utilize compounds that have the potential to be transported. Obviously drugs cannot be transported if they are degraded or insoluble. However, it is sometime critical to identify which compound in a homologous series has the best chance of being absorbed. Thus, early in drug discovery a key question concerns the membrane transport properties of drug candidates. The role of IAM chromatography thus resides early in the drug discovery process because drug partitioning to IAM columns can predict whether the compound has favorable transport properties (this assumes the compound is in solution). IAM chromatography is simple, accurate, and can provide key information about the potential transport properties of new compounds during the drug discovery process. In summary, the key concept being evaluated by IAM analysis refers to the question, "If a compound is in solution, will the compound be transported through biological membranes?"

Another limitation of the IAM method is that some compounds will be actively transported or transported by a paracellular pathway and IAM chromatography will not identify these compounds. To address the potential of identifying compounds that are actively transported, we are purifying membrane proteins responsible for peptide transport from intestinal cells with the intent of immobilizing these proteins on IAM surfaces for screening. In other words, immobilized membrane transporters may function as affinity columns for identifying high and low binding substrates of the transporter. IAM surfaces have been used to purify membrane proteins[20-23] and immobilize enzymes in functional forms.[24,25]

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(29) The ^{ether}IAM.PC$^{C10/C3}$ column was prepared by covalently immobilizing a single chain ether phosphatidylcholine (PC) ligand on silica propyl amine, followed by encapping with a long chain (decanoyl) anhydride (C10) and a short chain (propionyl) anhydride (C3).[12] It should be noted that more than 20 different IAM columns were prepared over the last 4 years.[9-15] To avoid confusion on which IAM column should be used for the purpose of drug screening, the manufacturer (Regis Technologies Inc.) has prepared an IAM.PC.DD column for drug discovery studies using IAM columns. In our previous work the IAM.PC.DD column was denoted as $^{\delta G}$IAM.PC$^{C10/C3}$.[6] Solute retention data on ^{ether}IAM.PC$^{C10/C3}$ columns are identical to solute retentions on $^{\delta G}$IAM.PC$^{C10/C3}$ columns.[6] Most of our IAM work for method development is performed on 12 μm particles, but to improve column efficiency, IAM.PC.DD columns are prepared from 5 μm silica particles.

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